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FURTHER OBSERVATIONS ON SUBCUTANEOUS ABSCESSES IN RABBITS

THE CARRIER STATE AND ITS RELATION TO RABBIT SEPTICEMIA *

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In a previous paper[1] I called attention to the not infrequent occurrence of well defined subcutaneous abscesses in rabbits. From these I was able to obtain often in pure culture a small gram-negative, nonmotile, coccoid bacillus with some tendency to form chains, which had some of the properties of B. influenzae, but in certain respects was distinctly different. This organism was isolated and grown on artificial mediums, and on subcutaneous injection into healthy rabbits it reproduced typical abscesses from which it again could be readily isolated. An identical organism was isolated from rabbits suffering or dying from respiratory infections, which not infrequently attacked in epidemic form the animals in the laboratory. These epidemics are commonly observed and are called by a variety of names, snuffles, rabbit influenza, 'Brustseuche,' rabbit septicemia, etc. Many such epidemics, if not all, are due to bacilli of this type, organisms which are classed in the hemorrhagic-septicemia group and are closely related to or probably identical[2] with such organisms as B. bovisepticus, B. suisepticus, B. avisepticus, etc.

Many workers have described bacteria of this general type as the causative agents in epidemics in rabbits, but there exists considerable disagreement in their descriptions of the bacilli. Furthermore, the clinical description of these outbreaks by various observers differs to some degree so that one can readily understand why they are usually considered as a group rather than a single variety. Laven,[3] for example, has tabulated the various bacteria that have been found as apparently causative organisms in epidemics in rabbits and guinea-

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[1] Jour. Infect. Dis., 1913, 12, p. 42.

[2] Besemer: Jour. Bact., 1917, 2, p. 77.

[3] Centralbl. f. Bakteriol., I, O., 1910, 54, p. 97.

pigs. Fourteen gram-negative bacteria have been described, all of which differ from each other in some detail. All, however, when injected into animals produce similar pathologic lesions.

However, since the work of Hüppe[4] the bacillus of rabbit snuffles or rabbit septicemia has come to be a definitely recognized bacillus belonging to the hemorrhagic-septicemia group, and these various organisms, or at least most of them, no doubt are strains belonging to this group, having acquired varying degrees of virulence and specific selective action for various animals,[5] and no doubt certain other modified characteristics.

Schimmelbusch and Mühsam[6] described a small organism isolated from abscesses in rabbits which agrees in essential details with this bacillus. No doubt they were dealing with the same organism but did not recognize that it belonged to the hemorrhagic-septicemia group of Hüppe. This bacillus is usually referred to in the literature independently[7], and so far as I can determine its relation to the hemorrhagic-septicemia bacillus has not been recognized heretofore. This is the only reference I have found of examinations made on these subcutaneous abscesses, and though the authors in this report considered the bacillus a specific causative organism, there is no doubt from their description that it belongs to the septicemia group.

Since my first article, which included data from 18 animals, I have observed and examined 12 or more similar abscesses in rabbits from which I have always been able to isolate the same small bacillus. The morphology of these strains agrees with that given in the first series that I studied. A point in their morphology that was brought out previously but may be again noted is the tendency of the bacillus, especially of certain strains, to produce thread forms quite like the influenza bacillus.

The organism yields its rather delicate, moist, somewhat slimy growth on blood agar or other mediums containing animal fluids for a few generations. After about the 3rd or 4th generation the growth may be very scant or fail completely even in rich blood agar. If the oxygen supply is limited by growing in a tube connected by rubber tubing with a 2nd tube inoculated with the hay bacillus, the growth is not decidedly enhanced.

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[4] Berl. klin. Wchnschr., 1886, 23, p. 753.

[5] Hutyra: Cited by Kolle and Wassermann, Handb. d. path. Mikroorg., Ed. 2, 1913, 6, p. 81.

[6] Arch. f. klin. Chir., 1896, 52, p. 564.

[7] Cited by Kolle and Wassermann, Handb. d. path. Mikroorg., 1913, 6, p. 181.

I have tested some of the strains recently isolated on the various sugars, as was done previously with other strains, and found that they agree in every detail. That is, they produce acid without gas-production in dextrose, saccharose, and mannite, but not in inulin, raffinose, salicin, maltose, or lactose. These reactions agree in every way, so far as they are comparable, with recent data given by Besemer[2] on the hemorrhagic-septicemia group.

The virulence of the infections varies considerably. I have observed epidemics in which the majority of the animals attacked died in a few days. On the other hand, the infection may be relatively mild and manifest itself by only a slight purulent discharge from the nose, or even by a trace of moisture about the nostrils. The variation in the virulence of the strains of this organism was readily demonstrated experimentally.

Two rabbits were injected intranasally with 1 c.c. of a broth culture of a strain isolated 3 weeks previously from the nose of a sick animal. One remained entirely well and the other developed a very slight discharge from the nose on the 2nd day. One week later both were again inoculated with 1 c.c. intranasally with another strain just isolated from the lung of an animal dead of the disease. One died in 2 days of general septicemia, and the other died in 3 days with typical fibrinous pericarditis, pleuritis, and peritonitis, and acute pneumonia and septicemia. Pure cultures of the bacilli were isolated from the blood and various organs.

Not all rabbits are equally susceptible. One animal resisted intranasal inoculation of 2 different strains which readily killed other rabbits; however on subcutaneous injection of 0.25 c.c. of 1 of these same strains, it died of septicemia in 48 hours.

Occasional outbreaks in the laboratory animals and the fact that the disease is practically endemic now in many laboratories and animal-breeding houses led me to note certain points in connection with carriers in this disease, a point which has not received much attention. Of the animals that do not die of the infection, a considerable number improve and the disease assumes a chronic form, in which state perhaps only a slight discharge or moisture about the nose continues.

I have had 5 animals under observation for many months. They were at first quite ill, but later improved. From the slight nasal discharge of these animals the small bacilli have been cultivated repeatedly during this period. One animal has now been under observation for about 11 months; the others 4-9 months. They are in good general condition, are not emaciated, and might easily pass for normal animals. One of them, which had been under observation for 4 months, developed a disease of the middle and internal ear, which caused him to rotate his head to the left. He was killed and there was found an otitis media with extensive abscess-formation containing thick grayish pus, which yielded a pure growth of the small bacillus. The mucosa of the nasal cavities was slightly inflamed, there being a slight nasal discharge.

Subcutaneous abscesses have not been observed in such animals unless the bacteria are injected under the skin, where they may form and remain for a long time. Such animals, therefore, are not immune to the bacilli even to their own strains. Autogenous organisms from the nose were injected subcutaneously into 3 animals with positive results, the animals dying of septicemia from doses of 0.5 c.c.

An attempt was made to find out how dangerous the animals with these chronic infections are to other animals.

In the first experiment 6 young healthy rabbits were put in the same cage with a rabbit having typical acute snuffles in the early stages, and with many typical bacilli in the discharge. To be sure of more intimate contact the noses of the normal rabbits were rubbed against the nose of the infected one. After 1 week none of the animals developed snuffles. Cultures from their nasal mucosa did not give positive results for the bacilli. Three of these animals were kept under observation for 2 weeks longer without developing the disease. The other 3 were now inoculated subcutaneously with 1 c.c. of a 24-hour culture of the bacilli. These all died in 3-5 days with typical symptoms and findings of septicemia.

Several similar experiments have been made by placing normal young animals in intimate contact with chronic nasal carriers described, but all these experiments have given negative results. In some cases the normal animals were permitted in the same cages with those infected for months (in 1 case, 7 months) and have remained healthy. Such contact animals do not apparently become carriers since the cultures made repeatedly from the nasal mucosa were always negative for the septicemia bacilli. From these experiments then it would seem that such chronically infected animals are not a source of danger to healthy animals by infecting them through the respiratory passages. However, the bacilli from these carriers, when injected subcutaneously, may give rise to typical abscesses, and if the dose is large enough septicemia will result. Through bites, therefore, and perhaps in other ways, these animals may be a source of danger. Every now and then it may be that a susceptible animal may acquire the disease through the respiratory tract, but no susceptible animals were detected in the experiments.

In addition to nasal carriers of the bacilli the animals which develop the subcutaneous abscesses also might be considered carriers of the disease. The abscesses are no doubt caused by infection through slight injuries received from the scratching or biting of an animal by one harboring a strain of the bacilli probably of reduced virulence in its mouth or on some other part. Experimentally, as noted, the injection of small doses of this organism reproduces the abscesses. It may not be necessary for the animal in question to be ill, since bacilli of this type have been shown by Fiocca and others[8] to occur at times in the mouth and nose of apparently normal animals, which might serve then as healthy carriers. The abscesses are a chronic form of the infection where the bacilli exist often in pure form for a long time. They may rupture on the surface and discharge their contents, but often they do not. Entire removal of the encapsulated mass leads to complete recovery, and excision with discharge of contents will at times lead to recovery, but often a new abscess or several abscesses may reform in the vicinity. Bacteriologically, they are striking examples of a chronic subcutaneous focus of infection persisting at times almost indefinitely.

Whether these abscesses are dangerous to other animals and play a rôle in the direct transmission of the disease is doubtful. The bacilli from these abscesses when inoculated into the nose in quantities of several cubic centimeters do not develop septicemia. In 2 animals injected with large doses a mild acute rhinitis developed, with slight watery discharge from the nose for several days. I have also produced a small pneumonic lesion in the lung, but the characteristic acute pleuropericarditis and pneumonia I have not been able to cause by intranasal injection. With sufficiently large doses administered subcutaneously such lesions as well as general peritonitis and septicemia are readily produced. No doubt then these bacilli are very inferior in virulence and invasive properties to the organism met with in the respiratory passages and internal organs in the acute disease. However, by passing the organisms through only 2 or 3 animals by subcutaneous injection and reisolation from the blood or pericardium, the virulence is markedly raised and compares with that found in the natural disease in this respect.

When the virulent septicemia bacillus remains in an abscess for a time it loses its virulence, as is strikingly shown in the following experiment.

A rabbit was inoculated subcutaneously with 0.1 c.c. of an organism which had killed 2 rabbits by intranasal injection. An abscess the size of a large walnut appeared at the site, and remained with little change for 5 months. The organism was then reisolated from it, and 2 c.c. of a culture was inoculated intravenously into 2 rabbits without any results. At the end of 3 days a 2nd inoculation was made again without result. Eleven days after the 1st inoculation the animals were given intranasal inoculation with a freshly isolated bacillus from a sick animal, and both succumbed in 36 hours with septicemia. Culturally and morphologically, it should be said, the bacillus had retained all its original properties during its 5 months' sojourn in the abscess.

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[8] Cited by Kolle and Wassermann, Handb. d. path. Mikroorg., 1913, 6, p. 90.

In connection with these experiments there was opportunity to make observations on the resistence of the infected rabbits to subsequent infection, both natural and artificial, and also to study immunity reactions, especially on animals with the abscesses.

In the first place a vaccine was made with several strains of this organism, and a series of rabbits were injected for purposes of immunization. The vaccine was prepared by growing the bacillus on heavy blood agar for 24-36 hours, and suspending in 0.8% salt solution. After heating to 60 C. for 30 minutes, which kills the bacilli, the suspension was diluted so that 1 c.c. contained 100,000,000. Injection was made subcutaneously into a series of 7 rabbits using 100,000,000 per kilogram.

After the 7th injection the immunity of this series of rabbits was tested by inoculating them subcutaneously in the back with small doses of a 24-hour ascitic-broth culture recently isolated from the nasal discharges of an infected rabbit, which later died of rabbit septicemia. Six control animals were likewise inoculated. In 5 of the immunized series the dosage was 1 c.c. of the broth culture per kilogram of rabbit weight. In the other 2 a very much smaller dose (1 loop in 1 c.c. of salt solution) was given. On the 3rd day one of the former and on the 4th day another animal died, there being found at necropsy extensive infiltration into the muscles of the back with invasion of the lungs and blood stream. Of the 6 control rabbits 2 died, one on the 6th day after inoculation, another 3 days after inoculation. The findings in these animals were quite like those in the immune series.

All of the other rabbits, both the immunized and the controls, developed characteristic subcutaneous abscesses at or near the site of inoculation. No appreciable differences were noted between the 2 sets of animals. Those receiving the very small dose of 1 loop also developed abscesses which matured decidedly slower than those resulting from the larger doses.

All of the animals receiving the live bacilli in the course of the following 4 or 5 days lost 200-300 gm. Those in both series that lived, notwithstanding the abscesses, recovered their former weights in 3-4 weeks.

These animals then continued to live many weeks, nearly all of them gaining in weight. Inasmuch as rabbit influenza not uncommonly breaks out in the laboratory, the animals were carefully observed for any such outbreak. One of the control rabbits died of some undetermined cause some weeks later. Three months after the inoculation and while the abscesses still persisted, 2 of the 3 control rabbits developed typical snuffles, and from the nasal discharges the baccilli were found almost pure. Also at this same time in 4 of the immunized series, typical snuffles appeared and from the nasal discharge the organism was grown practically pure. Both series ran an identical clinical course which was not very severe, and no deaths occurred. In the cage containing the 4 immunized rabbits 1 of the animals became ugly and repeatedly bit the other 3 in various parts of their bodies, especially about the eyes. These wounds suppurated and the eyes became badly infected, and in 2 animals were completely closed from the swelling and exudate. From these lesions the bacilli were readily cultured in large numbers mixed with a few staphylococci.

Evidently protection against experimental inoculation or against natural infection was not obtained by the vaccination or through the presence of abscesses. It has already been noted that even the carrier state does not protect against the development of typical abscesses on subcutaneous inoculation with small doses of the bacilli. Acute snuffles, however, did not develop in the 5 carrier rabbits while under observation, though they were repeatedly exposed.

In my previous paper I reported testing the serum for the presence of agglutinins in a number of rabbits having abscesses, of rabbits having snuffles and of guinea-pigs, after receiving several injections of dead bacilli. The results were all practically negative. I repeated the agglutination tests in the series of rabbits just referred to after they had been treated with 7 injections of dead bacilli. The results were practically negative, there being a trace of clumping only in dilutions of 1 : 20 in a few of the animals. Two strains, one isolated from the respiratory tract and the other from an abscess, were used.

Four of the animals of the immunized series that remained alive, after injection with living bacilli and abscess-production, developed snuffles which continued for several weeks. It was thought that these animals having received 7 doses of killed bacilli and having also the abscesses containing the live bacilli for several weeks and the respiratory infection for 4 weeks would show the antibodies, if any were formed against these bacilli. Agglutination tests made, however, even with autogenous strains, showed no appreciable clumping, either by microscopic or macroscopic methods.

With the serum of these same animals Dr. Moore, using the bacilli as antigens in varying concentration, was not able to detect any fixation of the complement.

The work of Chamberland and Jouan[9] on the interagglutination with the hemorrhagic-septicemia group was carried on with the serum of a highly immuned horse and showed with various strains from different animals including the rabbit, positive agglutination at dilutions varying from 1 : 10 to one to several thousand excepting with the homologous strain, where the agglutination appeared in dilutions of 1:60,000 to 1:80,000. These results, while clearly indicating the presence of antibodies in the immunized horse, are not directly comparable with the results given here.

In connection with some work carried out by Dr. Bernard Fantus on the problem of fever-production, several animals were treated with the heat-killed bacilli, and temperature and weight observations were made at suitable intervals.

The fever curve following such injections is quite definite and uniform, and occurs with a regularity that may make this organism

[9] Ann. de l'Inst. Pasteur, 1906, 20, p. 81.

a desirable agent for fever-production in the rabbit for experimental purposes. Fantus considers it the most reliable substance he has used and recommends it for testing the anti-pyretic action of drugs. Its fever-producing power is in all probability related to high degree of pathogenicity and virulence for the rabbit. There may be some difficulty encountered in producing this substance in bulk since the organism—at least certain strains—grows with considerable difficulty at times. By selecting suitable strains and using mediums rich in animal fluids this difficulty may be largely overcome.

The weight curve after the introduction into the rabbit of the dead organisms shows a typical and sharp decline followed by a more gradual rise. In the series of 8 rabbits injected with 100,000,000 per kilogram, following the 1st injection there was a drop in the weight curve of 200 : 300 gm. during the following week. Then usually appeared a more gradual return to the original weight, even though the animal received increasing doses at weekly intervals. Such animals apparently show an increasing tolerance to the repeated doses of this substance, which may be interpreted as a rough measure of an immunity reaction to the bacterial poisons. A similar phenomenon was noted in connection with fever-production.

SUMMARY

The well defined subcutaneous abscesses seen not infrequently in rabbits are caused by bacilli which are apparently identical with the rabbit septicemia bacillus. The virulence of the bacilli from the abscesses is markedly inferior to that of the organisms from the respiratory tract or internal organs.

On injecting a highly virulent bacillus in minute doses under the skin an abscess may form, remaining perhaps for many months. During this period the virulence of the bacilli is greatly decreased.

Rabbits after an attack of the disease and probably also without an attack become 'carriers' of the bacilli, both in the respiratory tract and subcutaneously in the abscesses. The virulence of the bacilli in these carriers is not high. However, organisms of low virulence of this group are known to rapidly increase their virulence under suitable conditions of transmission.

Rabbits treated with dead bacilli or having subcutaneous abscesses containing the bacilli are not immune to rabbit septicemia nor does their serum agglutinate the bacilli.

The heat killed bacilli on injection in suitable quantities into rabbits cause regularly a uniform fever curve.